Phytopathol. Mediterr. (2009) 48, 140–144

SHORT NOTE

Occurrence of the *Eutypa lata* sexual stage on grapevine in Rioja

ARGIÑE MURUAMENDIARAZ [1], PASCAL LECOMTE [2] and F. JAVIER LEGORBURU[1]

[1]NEIKER-Tecnalia, Basque Institute for Agriculture Research and Development, Apdo 46 , E-01080 VITORIA/GASTEIZ, Álava, Spain
[2] INRA UMR Santé Végétale Bordeaux-Aquitaine, ISVV, Boîte Postale 81, F-33883 Villenave d'Ornon Cedex, France

Summary. Eutypa dieback symptoms on grapevine have been reported in Rioja Alavesa since the nineteen eighties. However, attempts to find the perithecia of *Eutypa lata* in vineyards were not successful. Old vineyards in the Rioja Alavesa region were inspected for the presence of such perithecia. They were eventually detected in a 80-year-old Tempranillo vineyard in Laguardia. The morphology and size of the perithecia and the ascospores, as well as the colony features of the isolates on PDA were in agreement with those previously described for *E. lata*. The identification was confirmed by PCR using specific primers, as well as by sequence analysis of the rDNA ITS1/5.8S/ITS2 region. This is the first report of perithecia of *E. lata* in Rioja Alavesa and will improve understanding of the disease cycle of Eutypa dieback of grapevine in this region.

Key words: Eutypa dieback, life cycle, biology.

Eutypa dieback is a grapevine trunk disease. Foliar symptoms are dwarfed shoots with crinkled leaves. Wood symptoms are sectorial necroses of hard, darkened wood. The causal agent is *Eutypa lata* (Pers.: Fr.) Tul. and C. Tul. (syn. *E. armeniacae* Hansf. and M.V. Carter), a diatrypaceous fungus (Carter, 1991). Symptoms of Eutypa dieback have been observed in the Rioja Alavesa wine region (Basque Country, northern Spain) since the nineteen eighties (Lopez-Fernández, 1986). A previous survey for the foliar symptoms of Eutypa dieback in the Rioja Alavesa revealed that 11% of grapevines were affected with the disease (Mateo-Argomaniz, 1995).

Corresponding author: F.J. Legorburu
E-mail: jlegorburu@neiker.net
Fax: +34 945 281422

Although the climate in the Rioja Alavesa region is suitable for the development of perithecial stromata, they have not been found in the region, although its anamorph *Libertella blepharis*, which produces non-infectious conidia, is common. (López-Fernández and Sánchez-Monge, 1999). This way, in spite of the presence of typical symptoms of Eutypa dieback on grapevines in Rioja Alavesa, the occurrence of the perithecia of *E. lata* has remained uncertain. In addition, previous studies have suggested that botryosphaeriaceous fungi constitute the most common pathogens responsible for arm and trunk dieback in the nearby region of Castile-León (Úrbez-Torres *et al.*, 2006a; Martín and Cobos, 2007) and in Spain, as a whole (Armengol *et al.*, 2001). Since ascospores are necessary to complete the infective cycle of the fungus, the present study attempted to detect the perithecia of *E. lata* on grapevine.

While it is known what climatic conditions are favorable for the release of *E. lata* ascospores in various wine regions over the world (Paillassa, 1992; Ramos *et al.*, 1975; Trese *et al.*, 1980), less attention has been paid to the conditions conducive to perithecial stromata formation. Carter (1991) established a minimum annual rainfall threshold of 350 mm for perithecia formation. Ramos *et al.* (1975) reported abundant formation of perithecia on dead apricot wood when the annual rainfall was higher than 800 mm, while formation was sparse with an annual rainfall of ca. 330 mm and very rare when rainfall was below 280 mm. Trese *et al.* (1980) reported perithecia from areas in Michigan where rainfall was 775–825 mm. Perithecia of *E. lata* are well documented in the Bordeaux wine region, with its Atlantic climate and an annual rainfall of over 900 mm rainfall (Dubos, 2002). On the other hand, perithecia have not been clearly documented in Castilla y Leon and Rioja Alavesa with their Mediterranean climate (400–500 mm annual precipitation). Recent findings of perithecia on grapevine from Catalonia (North Eastern Spain) appeared to belong to other diatrypaceous genera after molecular analysis (J. Luque, pers. comm.). *E. lata* perithecia have however been reported from Extremadura in the Tierra de Barros wine region (western Spain) (Arias-Giralda, 1998) (Fig. 1).

In the present study, we inspected dead vine wood from old vineyards in Rioja Alavesa for *E. lata* perithecia. Stromata suspected of belonging to the fungus were collected, taken to the laboratory and inspected under a compound microscope. Ascospores were cultivated on potato-dextrose agar (PDA) amended with streptomycin, and the morphological features of the colonies were recorded. Pure cultures were obtained from the hyphal tips. Identification was confirmed by polymerase chain-reaction (PCR) analysis using the Lata1 and Lata 2-1 primers described by Lecomte *et al*. (2000). The rDNA ITS1/5.8S/ITS2 were amplified by PCR and sequenced. Sequence data were aligned and compared with those of 9 species belonging to the family Diatrypaceae (Acero *et al.*, 2004) using BioEdit software (Hall, 1999). A phylogenetic analysis was conducted using MEGA 3.1 software (Kumar *et al.*, 2004), with *Neurospora crassa* (Sordariaceae) as an outgroup.

Perithecia of *E. lata* were found in an 86-year-old Tempranillo vineyard located in Laguardia and showing 15% of diseased grapevines based on inspection of foliar symptoms. The morphology of both the perithecia (500–600 μm diameter) (Fig. 2A) and the ascospores (allantoid, yellowish, 8–9×1.5–2 μm) (Fig.2B), and the colony growth rate (2.56 cm in 5 days) were in agreement with those described for *E. lata* (Carter, 1991).

The size of the amplification product was between 350 bp and 400 bp after gel electrophoresis. The sequence of the amplicons of two isolates, 15H11 from an infected wood sample and MC3 from perithecia, were 100% homologous and contained 359 bp each. These two isolates were also grouped together with the referenced isolates of *E. lata* and *E. armeniacae,* with 97% bootstrap support (Fig. 3). While an evolutionary study of the isolates obtained is beyond the scope of the present work, this phylogenetic analysis discards any false positives that could occur with other Diatrypaceae and these primers (Rohlshausen *et al.*, 2004).

In this way, the occurrence of the sexual stage of *E. lata* in Rioja Alavesa was confirmed. The finding suggests that grapevine infection is likely to occur in this region. The occurrence of perithecia is epidemiologically important. At a regional level, the absence of perithecia would have implied that inoculum should move over long distances (Ramos *et al.*, 1975). On the scale of the individual vineyard, the presence or absence of perithecia is important to understand the spatial distribution of the disease (Munkvold *et al.,* 1993). This is not to say however that *E. lata* is rare in a Mediterranean climate. But this fungus cannot always find the conditions to reproduce sexually in such a climate. Of three research works on the pathogenicity of *E. lata*, only Péros and Berger (1999) reported perithecia from the French Midi, from which ascospore isolates were obtained. Isolates from Catalonian grapevines (Péros and Berger, 2003) and from Greek almond trees (Rumbos, 1985) were obtained from wood chips. A report of *E. lata* perithecia being found near Verona, Italy (Cortesi and Milgroom, 2001), really falls within the purview of the central European biogeographic region (Anonymous, 2007).

Studies in California have shown that *E. lata* favours cooler and wetter climates as compared with certain Botryosphaeriaceae spp. that are found in the warmer and drier desert regions of South California (Úrbez-Torres *et al.*, 2006b). However, the distribu-

tion of these species in grapevine cankers overlaps over wide areas of central and northern California (Úrbez-Torres *et al.*, 2006b). *A* similar phenomenon could also occur in Rioja Alavesa, where *Diplodia seriata* has recently been isolated (Muruamendiaraz and Legorburu, this issue). The relative importance of *E. lata* and *D. seriata* as the causal agents of grapevine dieback in this region deserves further study.

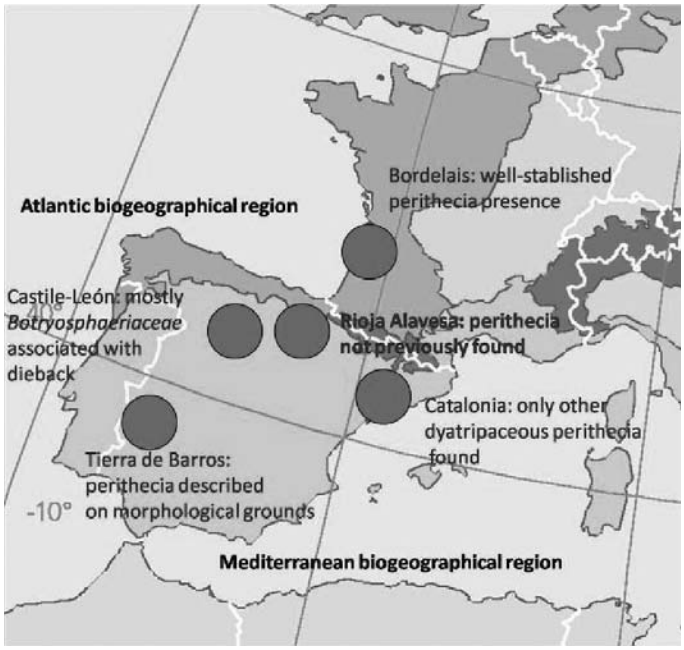

Fig. 1. Biogeographical regions of south western Europe (Anonymous, 2007) with the location of the wine regions mentioned in the text

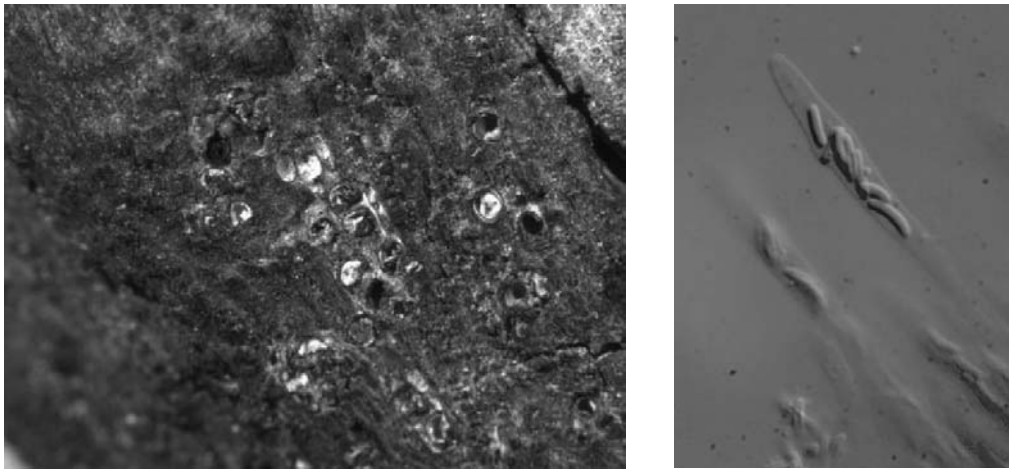

Fig. 2. Perithecia and ascospores of *Eutypa lata* collected in Laguardia.

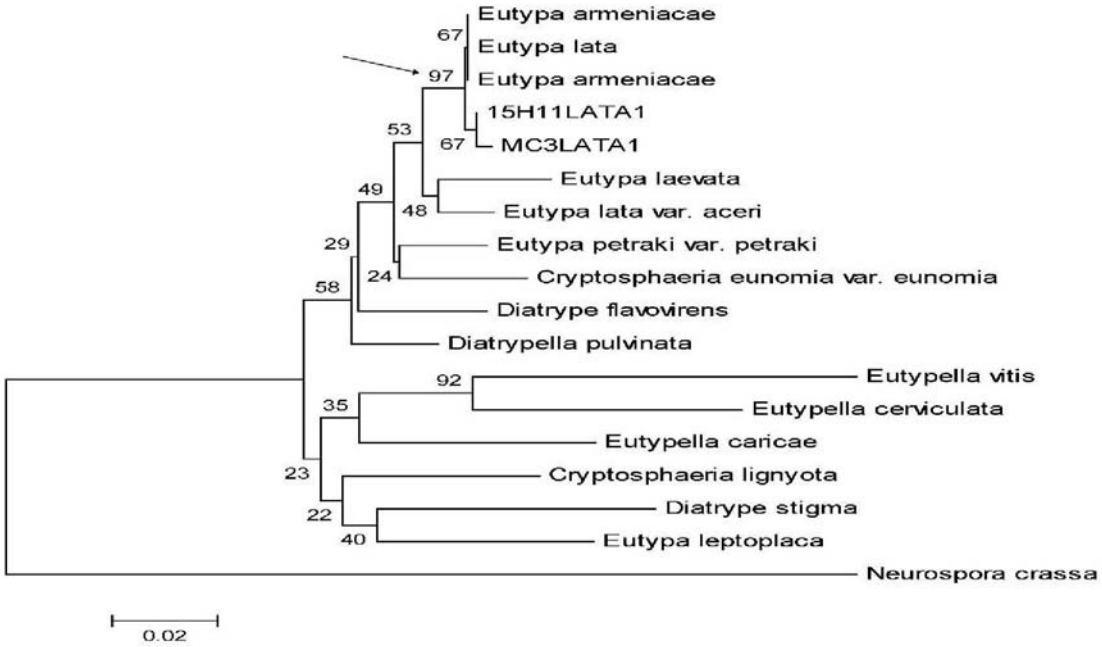

Fig. 3. Phylogenetic tree of the rDNA ITS1/ 5.8S/ITS2 region. Isolates 15H11 and MC3 were obtained from infected wood and ascospores respectively. GenBank accession numbers as in Acero *et al.* (2004) plus AY681193 for *N. crassa*.

Acknowledgements

Argiñe Muruamendiaraz is a PhD student funded by the Department of Agriculture, Fisheries and Food of the Basque Government. This work was supported by the Aquitaine-Euskadi Co-operation Fund and project RTA2007-00023-C04-02 from the Spanish National Institute for Agricultural and Food Research (INIA). The authors thank Manuel Mariño, Ernesto Ortiz and María-Eugenia Sanz for skilled technical assistance and the grape growers of the Alavese Farmer's Union (UAGA) for allowing free access to their vineyards.

Literature cited

Acero F.J., V. González, J. Sánchez-Ballesteros, V. Rubio, J. Checa, G.F. Bills, O. Salazar, G. Platas and F. Peláez, 2004. Molecular phylogenetic studies on the *Diatrypaceae* based on rDNA-ITS sequences. *Mycologia* 96, 249–259.

Anonymous, 2007. *European Forest Types. Categories and Types for Sustainable Forest Management Reporting and Policy*. 2nd edition. European Environment Agency, Copenhagen, Denmark.

Arias-Giralda A., 1998. Eutipiosis. In: *Los Parásitos de la Vid. Estrategias de Protección Razonada*, 4th edition. (G. Barrios i Sanromá, R. Castillo-López, R. Coscollá-Ramón, A. Lucas-Espadas, J. L. Pérez-Marín, J. Toledo-Paños, ed.), Mundiprensa, Madrid, Spain, 202–204..

Armengol J., A. Vicent, L. Torné, F. García-Figueres and J. García-Jiménez, 2001. Fungi associated with esca and grapevine declines in Spain: a three-year survey. *Phytopathologia Mediterranea* 40, 325–329.

Carter M.V., 1991. The status of *Eutypa lata* as a pathogen. *Phytopathological Paper* 32. International Mycological Institute. CAB International, Wallingford, England, 1–59.

Cortesi P. and M.G. Milgroom, 2001. Outcrossing and diversity of vegetative compatibility types in populations of *Eutypa lata* from grapevines. *Journal of Plant Pathology* 83, 79–86.

Dubos B., 2002. *Maladies Cryptogamiques de la Vigne*. 2ème édition. Féret, Bordeaux, France, 208 pp.

Hall T.A., 1999. BioEdit : a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41, 95– 98.

Kumar S., K. Tamura and M. Nei, 2004. MEGA3: an integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics* 5, 150–163.

Lecomte P., J.P. Péros, D. Blancard, N. Bastien and C. Délye, 2000. PCR assays that identify the grapevine dieback fungus *Eutypa lata*. *Applied and Environmental Microbiology* 66, 4475–4480.

López-Fernández E., 1986. Enfermedades de madera en el

viñedo de Rioja Alavesa. *Sustrai* 5, 19–20.
López-Fernández E. and M.A. Sánchez-Monge, 1999. Eutipiosis. In: *25 años de los premios mildiu. Jornada Técnica de Fitopatología en Viticultura*. November 1999, Logroño, Rioja, Spain, 31–42.
Martín M.T. and R. Cobos, 2007. Identification of fungi associated with grapevine decline in Castilla y León (Spain). *Phytopathologia Mediterranea* 46, 18–25.
Mateo-Argómaniz J., 1995. Incidencia de la Eutipiosis en el viñedo de Rioja Alavesa. *Phytoma – España* 66, 15–18.
Munkvold G.P., J.A. Duthie and J.J. Marois, 1993. Spatial patterns of grapevines with eutypa dieback in vineyards with or without perithecia. *Phytopathology* 83, 1440–1448.
Paillassa E., 1992. L'Eutypiose de la vigne (*Eutypa lata* (Pers.; Fr.) Tul.). Aspects épidemiologiques et leur application à la mise au point d'une méthode d'évaluation des fongicides *in vivo*. PhD Thesis. University of Bordeaux 2.
Péros J.P. and G. Berger, 1999. Diversity within natural progenies of the grapevine dieback fungus *Eutypa lata*. *Current Genetics* 36, 301–309.
Péros J.P. and G. Berger, 2003. Genetic structure and variation in aggressiveness in European and Australian populations of the grapevine dieback fungus, *Eutypa lata*. *European Journal of Plant Pathology* 109, 909–919.
Ramos D.E., W.J. Moller and H. English, 1975. Production and dispersal of ascospores of *Eutypa armeniaca* in California. *Phytopathology* 65, 1364–1371.
Rohlshausen P.E., F. Trouillas and W.D. Gubler, 2004. Identification of *Eutypa lata* by PCR-RFLP. *Plant Disease* 88, 925–929.
Rumbos I.C. 1985. Further pathogenicity studies of *Eutypa lata* (=*E. armeniacae*) on almond. *Options Méditerranéennes* 85, 79–89.
Trese A.T., C.L. Burton and D.C. Ramsdell, 1980. *Eutypa armeniacae* in Michigan vineyards: ascospore production and survival, host infection and fungal growth at low temperatures. *Phytopathology* 70, 788–793.
Úrbez-Torres J.R., W.D. Gubler, H. Peláez, Y. Santiago, C. Martín and C. Moreno, 2006a. Occurrence of *Botryosphaeria obtusa, B. dothidea* and *B. parva* associated with grapevine trunk diseases in Castilla y León region, Spain. *Plant Disease* 90, 835.
Úrbez-Torres J.R., G.M. Leavitt, T. M. Voegel and W.D. Gubler, 2006b. Identification and distribution of *Botryosphaeria* spp. associated with grapevine cankers in California. *Plant Disease* 90, 1490–1503.

Accepted for publication: February 23, 2009